SUMMARY STATEMENT

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Application Number: **1 R01 AI079729-01**

Principal Investigator

DRUSANO, GEORGE LOUIS MD

Applicant Organization: ORDWAY RESEARCH INSTITUTE, INC.

Review Group: **ZAI1 DDS-M (M1)**
National Institute of Allergy and Infectious Diseases Special Emphasis Panel
Pharmacological Approaches to Combating Antimicrobial Resistance

Meeting Date: **02/12/2008** ***RFA/PA:*** **AI07-025**
Council: **MAY 2008** ***PCC:*** **M51F BR**
Requested Start: **07/01/2008**

Project Title: **Resistance Suppression for Influenza Virus With Combination Chemotherapy**

SRG Action: **Priority Score: 192**
Human Subjects: **10-No human subjects involved**
Animal Subjects: **10-No live vertebrate animals involved for competing appl.**

Project Year	Direct Costs Requested	Estimated Total Cost
1	539,152	751,690
2	552,055	769,680
3	564,791	787,436
4	579,356	807,743
TOTAL	2,235,354	3,116,549

ADMINISTRATIVE BUDGET NOTE: The budget shown is the requested budget and has not been adjusted to reflect any recommendations made by reviewers. If an award is planned, the costs will be calculated by Institute grants management staff based on the recommendations outlined below in the COMMITTEE BUDGET RECOMMENDATIONS section.

BUDGETARY OVERLAP
COMMITTEE BUDGET RECOMMENDATIONS

RESUME AND SUMMARY OF DISCUSSION: This excellent R01 application entitled "Resistance Suppression for Influenza Virus with Combination Chemotherapy" was submitted by Ordway Research Institute (Albany, New York) in response to RFA 07-025 "Pharmacological Approaches to Combating Antimicrobial Resistance" with Dr. George Drusano as the Principal Investigator (PI). The overall objective of this application is to determine the optimal chemotherapy regimen that minimizes the morbidity and mortality due to an influenza pandemic. In Aim 1, they propose to meet this objective by optimizing a hollow fiber infection model (HFIM) to select for viral mutants that are resistant to adamantine and neurominidase inhibitors and are the same as naturally occurring resistant mutants. They will then use this *in vitro* model in Aims 2 and 3 to optimize dosing regimens for each drug individually as well as in combination, to suppress the emergence of resistance. The strengths of this application include the well-conceived and logical experimental plan that inc reases confidence in the success of the application. In addition, they provide relevant and extensive preliminary data that supports their overall objectives. The expertise of the PI and the rest of the research team and their extensive experience with the HFIM system and influenza is a major strength. But, enthusiasm is lessened because of concern over whether the PI has sufficient time to devote to this project since he lists over 75% effort on four currently funded projects. Some significant weaknesses are also noted. A major concern is that the proposed *in vitro* system does not adequately address the very high potential for recombination between various influenza strains during a pandemic. This could lead to mechanisms of resistance that are not predicted by the *in vitro* model and not suppressed by the dosing regimens developed using this model. Another concern is that the PI does not make a convincing case that chemotherapy will play a major role in dealing with an influenza pandemic. An effective vaccine remains the optimal way to confront an influenza outbreak and chemotherapy will likely have a minimal role. Thus, this model will likely have limited utility for treating a flu pandemic. This weakness is lessened somewhat because the data generated by this system could be more effective in dealing with other types of viral outbreaks. Based upon the evaluation of scientific and technical merit, this application received a numerical score of 192.

DESCRIPTION (provided by applicant): The advent of H5N1 influenza A Virus is a critical wake up call. We are overdue for a global pandemic of Influenza Virus caused by H5N1 or some other influenza A virus. Such a pandemic could cause a very large number of deaths worldwide and major morbidity and economic disruption. It is important to recognize that optimal chemotherapy directed at such a pandemic virus is critical to reduce the attendant mortality and morbidity. In Specific Aim #1, we propose to employ our novel hollow fiber infection model (HFIM) to demonstrate that we can rapidly select Influenza Virus clones that are resistant to either adamantine or neuraminidase inhibitors and that the mutations conferring resistance will be the same as those of naturally- occurring strains. Once the system is validated that it is a good surrogate for the clinical selection of resistant isolates, we can employ our HFIM to pursue Specific Aim #2, and identify the optimal dose and schedule of administration of these agents given as monotherapy to optimize viral suppression and suppress the emergence of resistance. This will be accomplished through dose ranging and dose fractionation experiments. It is important to identify optimal dose ranges for resistance suppression and viral turnover suppression for drugs alone, as pharmacological differences between agents may allow "pharmacokinetic mismatching" at certain times within the treatment period. Such mismatched times may be more permissive for resistance emergence, even in the face of combination chemotherapy. Therefore, it is important for each drug in any combination to be optimal or near-optimal for resistance suppression on its own. In Specific Aim #3, we will pursue optimizing the drugs in combination for resistance suppression. Little has been done in this regard. We have developed a mixture model approach that will allow simultaneous description of the effect of these antiviral compounds in combination on both the fully wild-type viral population as well as the viral subpopulation with resistance mutations. As previous work from our laboratory with bacteria has shown, these different pathogen

populations will be differentially affected by the drug pressure in combination. Our approach will be to design combination therapy experiments from data developed in the monotherapy experiments of Specific Aim #2. We will then perform combination therapy experiments with sixteen different combinations of drug doses. All these data (drug concentrations over time for both drugs, the effect on the total viral population over time, and the effect on the mutant viral population over time) will be simultaneously co-modeled employing our completely novel mathematical population mixture model. Obtaining robust point estimates of system parameters will allow design of regimens that are optimized in the combination for Influenza Virus resistance suppression. We are well overdue for a global pandemic of Influenza virus that could wreak havoc, causing considerable mortality, morbidity and economic dislocation. Anti-influenza chemotherapy is critical in protecting ourselves from such a pandemic. The goals of this application are to 1) demonstrate that our in vitro hollow fiber system produces resistant Influenza Virus that reflect the clinical circumstance when suboptimal drug exposures are given 2) identify optimal drug exposures that suppress resistance by Influenza Virus to a neuraminidase inhibitor and the adamantine amantadine 3) identify the best ways to use these agents in combination to prevent Influenza virus from emerging resistant.

CRITIQUE: The comments in the CRITIQUE section were prepared by the reviewers assigned to this application and are provided without significant modification or editing by staff. They are included to indicate the range of comments made during the discussion, and may not reflect the final outcome. The RESUME AND SUMMARY OF DISCUSSION section summarizes the final opinion of the committee after the discussion and is the basis for the assigned priority score.

CRITIQUE 1:

Overall Evaluation: This is a solid, well conceived application that builds on many years of work by this group in a variety of infectious disease areas using the novel Hollow Fiber Infection Model (HFIM). They have identified important pharmacologic parameters which predict optimal anti-infective doses and schedules for a variety of infections. The investigators intend to use the HFIM to 1) demonstrate that it is possible to rapidly select influenza virus clones that are resistant to adamantine or neuraminidase inhibitors and that the mutations conferring resistance will be the same as those of naturally-occurring strains, 2) identify optimal dose and schedule of administration of these agents given as monotherapy to optimize viral suppression and suppress emergence of resistance, and 3) optimize combinations of drugs for resistance suppression. This group has good preliminary data using one influenza A strain (A/Albany/1/98) and has extensive experience using the HFIM in many pathogens (bacteria, fungi, and viruses). This model has been able to identify optimal PD-linked variables with respect to viral infection and resistance development because it is highly controlled. In this application, the investigators propose to extend the use of the HFIM using amantidine and oseltamivir to several H1N1/H3N2 influenza A viruses, the recombinant virus (rgA/Vietnam/1203/2004 x A/PR/8/34 (a surrogate for H5N1) and type B viruses. The principal investigator and co-investigators are well suited to conduct the proposed work using HFIM given their long history of work with bacteria, fungi, and HIV models.

Significance: With the widespread recognition of amantidine resistant influenza and increasing reports of neuraminidase resistant influenza, it is critical to gain an improved understanding of how the use and potential use of anti-viral medications can impact emerging resistance on treatment.

Approach: The proposed approach appears sound. The potential problems and mitigation strategies are well conceived. The timelines proposed to complete the three specific aims appear to be well founded given the extensive experience with the HFIM.

Innovation: It is encouraging that these investigators have been able to validate predicted doses and/or schedules with clinical data for other antivirals (stavudine, atazanavir, amprenavier, abacavir and GW420867X) for HIV. The approach to modeling combination chemotherapy is stated as completely

innovative and the approach to modeling resistance emergence and not cell kill is wholly novel. The ability to bridge this information to man by Monte Carlo simulation is particularly encouraging.

This group has preliminary model development data using HFIM for amantadine. In particular, this group has: Identified the optimal mixture of infected and uninfected MDCK cells, have grown viral stocks from over 20 clinical isolates and strains of influenza A and B, identified the EC50 for amantidine and oseltamivir for a low passage clinical isolate influenza A (A/Albany/1/98), described the growth characteristics of influenza A in the HFIM using MDCK cells. They have identified an optimal virus-infected to virus-uninfected ratio to determine the effect of drugs in the replicating HFIM system and evaluated the effects of amantadine treatment on viral load and resistance selection for A/Albany/1/98 (H3N2) [an amantidine-sensitive isolate] by continuous infusions of amantidine and analysis of drug concentrations, viral load (plaque assay) assessments, and gene sequence analysis.

They have demonstrated that amantidine doses suppressed viral replication compared with no drug control at 48 hours but not at subsequent time points, suggesting amantidine resistant virus had been produced. Found that an intermediate concentration of amantidine (0.8 mcg/mL) was associated with the highest peak viral titer suggesting that there may be an optimal concentration of selection of drug resistant mutant.

They have found no mutations in the M2 gene in the no-drug arm and found that mutations in the M2 gene were identified in all of the amantidine arms within 48 to 72 hours. Most were identical to those associated with clinical resistance (e.g., V27A, A30T, and S31N). Interestingly, the type of mutation was strongly associated with the dose of amantidine: 0.3 mcg/mL= 100% S31N, 0.8 mcg/mL = mixture of V27A and A30T), 2 mcg/mL = 100% were I32S and 6 mcg/mL = 100% were V27A.

They have found a good correlation between viral load and the fraction of mutants in the population. The highest to lowest viral load at 96 hours was 0.8>2.0>0.3> 6 mcg/mL. This order was also seen with the percentage of mutants in the population with 0.8 (60%) being the highest and 6 mcg/mL (0%) having the lowest.

This group also has preliminary model development data using HFIM for oseltamivir carboxylate. In particular, this group has used A/Syndey/5/97 strain in MDCK cells and a continuous infusion model and demonstrated that all doses of drug (except the 5 mcg/mL dose) inhibited viral replication to the same degree at 48 hours. At 72 hours, the HF unit treated with the 10 mcg/mL dose produced the most virus.

Investigators: Dr. Drusano, the PI, his co-investigators and collaborators are all highly experienced with pharmacodynamic models and mathematical models needed to complete the proposed work.

Environment: The resources and environments at Ordway Research Institute are clearly sufficient for the proposed research as are the resources at TGEN-North (to perform resistance testing), and the Center for Computational Bioscience.

CRITIQUE 2:

Significance: A hollow fiber infection model is to be used to provide information on resistance selection and to determine optimal dosage and frequency schedules for antiviral compounds to minimize emergence of resistant strains of influenza virus. In addition, combination therapies will be tested for optimal interactions. However, chemotherapy has a very limited role to play overall in influenza virus infections, which limits the value of this application. However, the approach may be applicable to other viral infections. Another concern is that the PI doesn't not sufficiently account for the likelihood of recombination between viruses in the "wild" during a flu epidemic and the possibility that the dosing regimens found using the HFIM will not adequately suppress resistance in these recombinant strains.

Innovation: The project and HFIM are very innovative.

Investigators: The investigators are very good but have limited experience with antivirals.

Environment: The environment is adequate.

CRITIQUE 3:

Overall Evaluation: This is an excellent, well-thought out application from a research group with outstanding expertise in the field of infectious diseases. This group has worked with the Hollow Fiber Infection Model (HFIM) for a long time to determine the optimal dosing regimens for a number of antibiotics, antifungals, and antivirals. In this application, the group will be evaluating the potential use of combination antivirals for the prevention of resistance in influenza.

Significance: Influenza resistance is increasing for currently available antivirals (amantidine and neuraminidase inhibitors). Therefore this study would provide insight into the potential use of combination therapy to prevent resistance. However, the use of antivirals in the treatment of influenza infections is still limited. Therefore, the value of this proposed study is decreased.

Approach: The approach appears to be sound. The investigators have a great deal of experience working with the HFIM.

Innovation: This study is very innovative in using combination therapy for the treatment of influenza and in modeling the emergence of resistance.

Investigators: The investigators (PI, co-investigators, and collaborators) in this study are well experienced in the area of pharmacodynamic and mathematical modeling to complete this work.

Environment: The resources and environment are adequate to perform this study.

THE FOLLOWING RESUME SECTIONS WERE PREPARED BY THE SCIENTIFIC REVIEW ADMINISTRATOR TO SUMMARIZE THE OUTCOME OF DISCUSSIONS OF THE REVIEW COMMITTEE ON THE FOLLOWING ISSUES:

PROTECTION OF HUMAN SUBJECTS (Resume): Not applicable.

VERTEBRATE ANIMALS (Resume): ACCEPTABLE. The use of a neutropenic mouse model was discussed and the animal welfare protections found to be acceptable.

BIOHAZARD COMMENT: Potential biohazards were discussed and the protections found to be acceptable.

DATA SHARING PLAN: The data sharing plan was discussed and found to be acceptable.

BUDGETARY OVERLAP: The committee expressed some concern over a potential overlap between this application and a similar application submitted by the PI and work already funded by other organizations.

COMMITTEE BUDGET RECOMMENDATION: The budget was recommended as requested with the following concern. The PI lists over >75% effort on four other currently funded projects. It is not clear whether he has sufficient time to devote an additional 30% effort (3.6 months) to this project. The PI's effort might need to be decreased and the budget adjusted accordingly.

NOTICE: The NIH has modified its policy regarding the receipt of amended applications. Detailed information can be found by accessing the following URL address: http://grants.nih.gov/grants/policy/amendedapps.htm

NIH announced implementation of Modular Research Grants in the December 18, 1998 issue of the NIH Guide to Grants and Contracts. The main feature of this concept is that grant applications (R01, R03, R21, R15) will request direct costs in $25,000 modules, without budget detail for individual categories. Further information can be obtained from the Modular Grants Web site at http://grants.nih.gov/grants/funding/modular/modular.htm

MEETING ROSTER

National Institute of Allergy and Infectious Diseases Special Emphasis Panel
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES
Pharmacological Approaches to Combating Antimicrobial Resistance
ZAI1 DDS-M (M1)
February 12, 2008 - February 13, 2008

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Consultants are required to absent themselves from the room during the review of any application if their presence would constitute or appear to constitute a conflict of interest.